

Serial No.: 09/902,481

Filed: July 9, 2001

allelic variations; that is, an amino acid sequence or a nucleotide sequence that usually has not been intentionally modified. Accordingly, by "non-naturally occurring" or "synthetic" or "recombinant" or grammatical equivalents thereof, herein is meant an amino acid sequence or a nucleotide sequence that is not found in nature; that is, an amino acid sequence or a nucleotide sequence that usually has been intentionally modified. It is understood that once a recombinant nucleic acid is made and reintroduced into a host cell or organism, it will replicate non-recombinantly, i.e., using the *in vivo* cellular machinery of the host cell rather than *in vitro* manipulations, however, such nucleic acids, once produced recombinantly, although subsequently replicated non-recombinantly, are still considered recombinant for the purpose of the invention. A representative amino acid sequences of a naturally occurring human integrin is shown in Figure 1F (SEQ ID NO:1). It should be noted that unless otherwise stated, all positional numbering of integrin proteins and integrin nucleic acids is based on these sequences (with position 1 equivalent to position 17 of Fig.1F). That is, as will be appreciated by those in the art, an alignment of integrin proteins can be done using standard programs, as is outlined below, with the identification of "equivalent" positions between the two proteins. Thus, the variant integrin proteins and nucleic acids of the invention are non-naturally occurring; that is, they do not exist in nature.—

A 2
cancel

Please replace paragraph beginning at page 31, line 20, with the following rewritten paragraph:

— In a preferred embodiment the variant integrin proteins of the invention will have a sequence that differs from a wild-type human integrin protein in at least three amino acid position selected from any of the positions in table 1.—

A 3

Please replace paragraph beginning at page 31, line 37, with the following rewritten paragraph:

— In a more preferred embodiment, the actual amino acid characteristics of each of the above possible positional mutants is defined in table 1 (SEQ ID NOS:3-6).—

A 4

S rial No.: 09/902,481

Filed: July 9, 2001

Please replace paragraph beginning at page 53, line 27, with the following rewritten paragraph:

AS
– In a preferred embodiment, the fusion partner is a stability sequence to confer stability to the library member or the nucleic acid encoding it. Thus, for example, peptides may be stabilized by the incorporation of glycines after the initiation methionine (MG or MGG0), for protection of the peptide to ubiquitination as per Varshavsky's N-End Rule, thus conferring long half-life in the cytoplasm. Similarly, two prolines at the C-terminus impart peptides that are largely resistant to carboxypeptidase action. The presence of two glycines prior to the prolines impart both flexibility and prevent structure initiating events in the di-proline to be propagated into the candidate peptide structure. Thus, preferred stability sequences are as follows: $MG(X)_nGGPP$ (SEQ ID NO:7), where X is any amino acid and n is an integer of at least four.

Please replace the paragraph beginning at page 72, line 7, with the following rewritten paragraph:

Q6
– Four mutant sequences (SEQ ID NOS:3-6) each were computed based on the open 1ido structure and the closed 1jlm structure using two different solvation potentials and subsets of core residues. Three out of a total of four designed ido mutants were well expressed; all have unique amino acid substitutions (Table 1). Fewer substitutions were predicted for jlm mutants, and only one of these, jlm2r (SEQ ID NO:6), was tested. All mutated sidechains are buried in the core of the I domain and are distant from the MIDAS and from the residues critical for iC3b binding (Li, R., et al. J. Cell Biol. 143:1523-1534 (1998); Zhang, L. & Plow, E.F. Biochemistry 38:8064-8071 (1999)), which are located on the top of the I-domain (Figure 1). Thus, the mutated residues cannot directly affect binding of iC3b. The energies of the selected sequences were determined in both the 1ido and 1jlm backbones (Table 1). All of the mutant sequences had energies that were lower than wild-type in the desired backbone configuration, and higher than wild-type in the undesired configuration. Thus, the open ido1q, 1do1r, and ido2r mutants (SEQ ID NOS:3-5) both stabilized the alphaM I domain in the 1ido conformation and destabilized it in the 1jlm

S rial No.: 09/902,481

Filed: July 9, 2001

conformation(Harbury, et al. Science 282:1462-1467 (1998)). Similar results were obtained regardless of the solvation potential used in the calculation. The energy of the wild-type sequence was lower in the 1jlm structure than in the 1ido structure, and thus the wild-type sequence should favor the 1jlm conformation (Table 1). This is consistent with the finding that for all alphaM, alphaL, alpha2 and alpha1 I-domain crystal structures determined to date, the I domain assumes a closed, 1jlm-like structure in the absence of a bound ligand or pseudo-ligand(Lee, et al., Cell 80:631-638 (1995); Qu, A. & Leahy, D.J. Proc. Natl. Acad. Sci. U.S.A. 92:10277-10281 (1995); Qu, A. & Leahy, D.J. Structure 4:931-942 (1996); Emsley, et al., J. Biol. Chem. 272:28512-28517 (1997); Baldwin, E.T. et al. Structure 6:923-935 (1998); Nolte, M. et al. FEBS Lett. 452:379-385 (1999); Rich, R.L. et al. J. Biol. Chem. 274:24906-24913 (1999)).-

On page 75, immediately preceding the heading "CLAIMS", please insert the enclosed text

entitled "SEQUENCE LISTING".

REMARKS

The specification has been amended to include a Sequence Listing and proper reference to the sequences therein and to correct minor typographical errors. Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

Entry of this amendment is respectfully requested. The amendments are made in adherence with 37 C.F.R. § 1.821-1.825. This amendment is accompanied by a floppy disk containing the above named sequence, SEQUENCE ID NUMBERS1-6 in computer readable form, and a paper copy of the sequence information. The computer readable sequence listing was